

## Reduced Seed Dispersal Effectiveness of the Large-Seeded Tree *Myrica rubra* in the Absence of Japanese Macaque

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### Introduction

Seed dispersal is a critical stage in a plant's reproductive cycle for the purpose of establishing new populations. The process of seed dispersal not only enhances the fitness of the maternal plant (reviewed by Howe and Smallwood 1982; Willson and Traveset 2000), but also has a direct effect on the genetic structure of plant populations (Hamrick et al. 1993). Diaspores have many adaptations that enhance long-distance seed dispersal by using vectors such as wind, water, and animals. However, a large proportion of plants in most communities are dispersed by animals (Howe and Smallwood 1982). Therefore, seed dispersal by animals is considered to play an important role in the genetic structure of plant populations and the maintenance of biodiversity within forests.

Large frugivores are effective seed dispersers quantitatively, but they are extremely sensitive to habitat modifications caused by human activity. If plant species depend only on large frugivorous animals for seed dispersal, the disappearance of these animals because of human activity could lead to a failure in seed dispersal. A two-step approach may be used to assess the effect of human activity on seed dispersal: compare seed dispersal with and without the aid of large frugivores and determine what type of effect the extinction of these frugivores would have on the dispersal of plant populations.

We examined Japanese macaque (*Macaca fuscata*) as a large seed disperser of *Myrica rubra*, a large-seeded tree species. Potential seed dispersers of *M. rubra* may be limited because the fruit and seed sizes of *M. rubra* are large (fruit: 13.2 mm in diameter, seed: 7.7 mm in diameter) and small frugivores cannot swallow these seeds. To evaluate the effectiveness of seed dispersal by Japanese macaque, we compared the seed dispersal of *Myrica rubra* in two forests, one with and one without Japanese macaque. We also examined the seed dispersal of *M. rubra* using genetic analysis of seeds found in the feces of macaques.

### Materials & Methods

#### *Study site*

The study sites were located in warm temperate evergreen forests on Yakushima Island (30°20' N, 130°30' E) and Tanegashima Island (30°43' N, 130°58' E), located in southwest Japan. Both islands shared similar

low-altitude broad-leaved evergreen forests. On the western part of Yakushima Island, a subspecies of Japanese macaque (*Macaca fuscata yakui*) lived in social groups of 20–30 individuals in the wild, without provisioning, in a protected National Park. Furthermore, these macaques had been habituated to observers and their social and feeding ecology had been studied continuously since 1976 (Maruhashi 1980; Yumoto et al. 1998; Hanya 2003). In contrast, Japanese macaque is thought to have been extirpated from Tanegashima Island for approximately 80 years as a result of human activity.

### ***Study species***

*Myrica rubra* (Myricaceae) was a common dioecious evergreen tree on both islands. It produces a large amount of fleshy fruit from late May to late June, and the fruits are an important food source for frugivores, especially for Japanese macaque on Yakushima Island. Japanese macaque is a local seed disperser of *M. rubra*, along with some bird species. Because the Japanese macaque has cheek pouches (i.e., a pocket in the cheek that opens into the mouth), it can store various fruits and seeds and travel long distances before choosing to either spit out or swallow the seeds (Yumoto et al. 1998).

### ***Comparison of fruit consumption with and without macaques***

To collect information on frugivorous tree visitors, observations were carried out on one tree by a single observer on Yakushima Island (with macaques) and on Tanegashima Island (without macaques; total observation times: Yakushima Island 73 h 46 min, Tanegashima Island 63 h 44 min). Observations were conducted from 600 to 1800, except during rainy periods. All visitors to the focal trees and all species observed around the focal trees were recorded. The visiting and feeding times of each visitor were also noted. If direct observation was possible, the number of fruits consumed per minute was recorded. We calculated the number of fruits consumed by each visitor species per visit using data on the feeding period per visit and the number of fruits consumed per minute by each visitor species. Finally, we estimated the total number of consumed fruits on each tree per day using the average data of the number of fruits consumed by each visitor species per visit and the number of visitors per day.

### ***Development of microsatellite markers for maternal analysis***

To conduct a maternal analysis of seeds dispersed by macaques under natural conditions, we developed 13 microsatellite markers from a genomic library enriched for dinucleotide (CT) repeats in the dioecious evergreen tree *M. rubra* and screened loci from 32 adult trees (Terakawa et al. 2006).

### ***Maternal analysis of seeds dispersed by macaques using microsatellite markers***

To collect macaque feces, we followed a single habituated troop of Japanese macaque living in the evergreen forest on the western part of Yakushima Island from 600 to 1800 during three sessions in 2005: 25–30 May (late May), 4–6 June (early June), and 17–20 June (late June). This troop comprised 26 individuals: seven

adult males, seven adult females, and 12 young individuals (E troop; Nishikawa, unpublished data).

We identified the maternal origins of dispersed seeds using the multilocus genotypes of 10 microsatellite loci of the seed endocarp. The genotype of endocarp tissue is identical to the genotype of the maternal tree because the endocarp is derived from the mother (Godoy and Jordano 2001). We determined the variation in maternal origin of dispersed seeds of *M. rubra* in feces of Japanese macaque during the fruiting period of *M. rubra* using the diversity and overlap index proposed by Grivet et al. (2005) as an index of the probability of maternal identity within and between gene pools. The diversity index of the maternal origin of dispersed seed ranges from 0 = highest diversity to 1 = lowest diversity. The overlap index of the maternal origin of dispersed seed ranges from 0 = lowest degree of overlap to 1 = highest degree of overlap. The inverse of the diversity index indicated the effective number of seed maternal origins; we calculated this for each period.

## Results

### *Comparison of fruit consumption with and without macaques*

We observed 25 species around the focal trees of *M. rubra* (Yakushima Island: Japanese macaque and 13 bird species; Tanegashima Island: 21 bird species). Six of the observed species were frugivores and five species were common to both islands. On Yakushima Island, the principal visitors of *M. rubra* were Japanese macaque and bulbul (*Hypsipetes amaurotis*; mean  $\pm$  standard error number of visitors per day: macaque,  $4.7 \pm 1.4$ ; bulbul,  $3.2 \pm 0.9$ ; other birds,  $0.3 \pm 0.2$ ). On Tanegashima Island, the principal visitor was bulbul (number of visitors per day: bulbul,  $3.8 \pm 0.9$ ; other birds,  $0.2 \pm 0.1$ ). There was no difference in the number of visits by bulbul between the two islands (*U*-test,  $P > 0.05$ ).

The feeding time per visit of each visitor species differed significantly on Yakushima Island (mean  $\pm$  standard error minutes per visit: macaque,  $10.8 \pm 1.0$ ; bulbul,  $0.8 \pm 0.1$ ; *U*-test,  $P < 0.001$ ). However, there was no difference in the feeding time per visit for bulbul between the two islands ( $0.5 \pm 0.1$  min per visit; *U*-test,  $P > 0.05$ ). The number of fruits consumed per minute by the macaques ( $14.0 \pm 2.4$ ) was greater than that by bulbul ( $5.5 \pm 1.3$ ). The number of fruits consumed per visit by the macaques was more than 30 times that by bulbul (Yakushima Island: macaque,  $151.9 \pm 13.8$ ; bulbul,  $4.3 \pm 0.6$ ; Tanegashima Island: bulbul,  $3.0 \pm 0.6$ ). In summary, the number of fruits consumed per day per tree on Yakushima Island (all, 730.7; macaque, 716.9; bulbul, 13.8) was greater than on Tanegashima Island (all, 11.4; bulbul, 11.4).

### *Development of microsatellite markers for maternal analysis*

The number of alleles of the microsatellite markers ranged from 2 to 14, and the expected heterozygosity ranged from 0.324 to 0.884 (Table 1). The total paternity exclusionary power when the mother was known was 0.99965. We tested linkage disequilibrium (LD) using Genepop version 3.4 software (Raymond and Rousset 1995). Three pairs of loci (my0043 and my0186, my0472 and my0792, and my0472 and my0793) showed significant LD in 78 pairwise tests with 13 loci ( $P < 0.05$ ). These loci allow mating system and parentage analyses to be carried out for *M. rubra*, thereby supporting studies of *M. rubra* seed dispersal by

macaques.

#### ***Genetic analysis of seed dispersal by macaques***

We collected 89 feces in total: 19 in late May, 20 in early June, and 50 in late June. For all periods, the average number of seeds per feces was  $8.56 (\pm 1.11 \text{ SE})$  with a range of 0 to 48. This number increased slightly between late May ( $9.26 \pm 2.22 \text{ SE}$ ) and early June ( $15.95 \pm 3.25 \text{ SE}$ ) and decreased significantly from early June to late June ( $5.34 \pm 0.96$ ). These changes in the number of seeds per feces in relation to the study period were statistically significant (Kruskall Wallis test:  $H = 12.248$ ,  $P = 0.002$ ), with a significant difference between early June and late June (Steel-Dwass test:  $t = 3.251$ ,  $P = 0.003$ ).

After genotyping, we identified 111 different maternal genotypes from 360 seeds in 41 feces (late May: 28 mothers from 106 seeds in 13 feces; early June: 52 mothers from 168 seeds in 11 feces; late June: 44 mothers from 86 seeds in 17 feces). The average number of different maternal genotypes per feces was  $3.85 (\pm 0.40 \text{ SE})$ , with a range of 2 to 11. Thus, macaques can disperse seeds from several different mother trees at a time (Fig. 1).

The average diversity of seed maternal origin per feces was very high (0.298) throughout the study period and increased from late May (0.425) to late June (0.239; 0.249 for early June). The total effective number of seed maternal origins in each period increased from late May (8.083) to late June (28.667).

Twenty-four maternal genotypes were shared between 92 pairs of feces of the 820 possible pairs (Fig. 1). Therefore, the seed maternal origin overlap among feces was very low (0.017). The seed maternal origin overlap among feces in each period decreased from late May (0.080) to late June (0.008).

## **Discussion**

#### ***Comparison of fruit consumption with and without macaques***

*M. rubra* seeds were dispersed by macaques and bulbul, and the disappearance of macaques could lead to a decrease in the number of seeds dispersed from mother trees. We observed a white-eye (*Zosterops japonicus*, gape size: 6.1 mm; Noma and Yumoto 1997) swallowing one red fruit of *M. rubra*. Bulbul and white-eye typically eat fruits, but capture insects to feed their offspring in the breeding season. It is difficult for frugivorous birds on Tanegashima Island to fill the role in seed dispersal that macaques fill on Yakushima Island. This suggests that the loss of macaques may significantly affect seed dispersal, producing a gap that other frugivores may not be able to fill. Furthermore, the loss of seed dispersers as a result of human activity may affect tree species that produce sap fruits.

#### ***Seed dispersal of *M. rubra* by macaques on Yakushima Island***

Macaques could disperse seeds from several maternal origins at the same time. Considering that the diversity of the maternal origin of *M. rubra* seed within feces was very high, macaques might efficiently enhance gene flow among local patches and populations of *M. rubra* and favor the settlement and growth populations with

high genetic diversity. Seed dispersal is a critical process affecting genetic structure in plant populations (Hamrick et al. 1993). However, the seed dispersal range would still be limited by the macaque's home range; seed dispersal by macaques may play an important role the population genetic diversity of *M. rubra*.

Our results suggest that the feeding behavior of macaques determines the seed dispersal pattern of *M. rubra* on Yakushima Island. The number of seeds and maternal diversity of seeds within feces, and the maternal overlap of seeds among feces were different in each sampling period. Macaques changed their feeding behavior during the fruiting of *M. rubra* to conform to food availability (Agetsuma and Noma 1995).

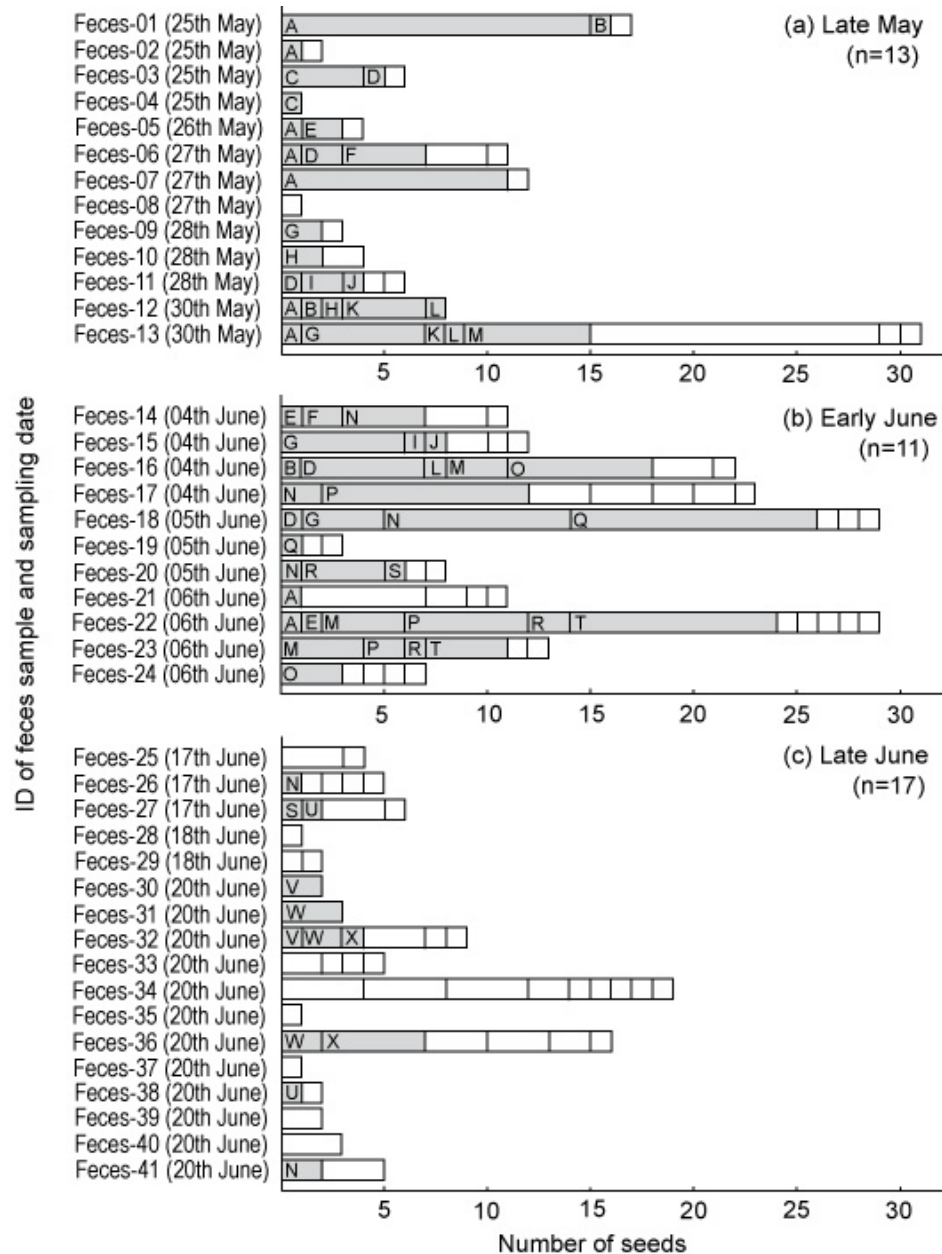
## References

- Agetsuma N, Noma N (1995) Rapid shifting of foraging pattern by Yakushima macaques (*Macaca fuscata yakui*) as a reaction to heavy fruiting of *Myrica rubra*. *Int J Primatol* 16: 247–260.
- Godoy JA, Jordano P (2001) Seed dispersal by animals: exact identification of source trees with endocarp DNA microsatellites. *Mol Ecol* 10: 2275–2283.
- Grivet D, Smouse PE, Sork VL (2005) A novel approach to an old problem: tracking dispersed seeds. *Mol Ecol* 14: 3585–3595.
- Hamrick JL, Murawski DA, Nason JD (1993) The influence of seed dispersal mechanisms on the genetic structure of tropical tree populations. *Vegetatio* 107/108: 281–297.
- Hanya G (2003) Age differences in food intake and dietary selection of wild male Japanese macaques. *Primates* 44: 333–339.
- Howe HF, Smallwood J (1982) Ecology of seed dispersal. *Ann Rev Ecol Syst* 13: 201–228.
- Maruhashi T (1980) Feeding behavior and diet of the Japanese monkey (*Macaca fuscata yakui*) on Yakushima Island, Japan. *Primates* 21: 141–160.
- Noma N, Yumoto T (1997) Fruiting phenology of animal-dispersed plants in response to winter migration of frugivores in a warm temperate forest on Yakushima Island, Japan. *Ecol Res* 12: 119–129.
- Raymond M, Rousset F (1995) Genepop (version 1.2): population genetics software for exact tests and ecumenicism. *J Heredity* 86: 248–249.
- Terakawa M, Kikuchi S, Kanetani S, Matsui K, Yumoto T, Yoshimaru H (2006) Characterization of 13 polymorphic microsatellite loci for an evergreen tree, *Myrica rubra*. *Mol Ecol Notes* 6: 709–711.
- Willson MF, Traveset A (2000) The ecology of seed dispersal. pp. 85–110. In: *Seeds: The ecology of regeneration in plant communities*. 2nd. Edition. M Fenner (ed.). CAB International, Wallingford, UK. [Reprint]
- Yumoto T, Noma N, Maruhashi T (1998) Cheek-pouch dispersal of seeds by Japanese monkeys (*Macaca fuscata yakui*) on Yakushima Island, Japan. *Primates* 39: 325–338.

**Table 1.** Microsatellite loci for *Myrica rubra*, including primer sequences, repeat motifs, annealing temperatures ( $T_a$ ), size ranges (bp), number of alleles detected ( $A$ ), number of genotyped individuals ( $N$ ), observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_E$ ) and fixation index ( $F$ ). DNA Database of Japan accession numbers are listed in parentheses under each locus.

Locus	Primer sequence (5'-3')	Repeat motif	$T_a$ (°C)	Size (bp)	$A$	$N$	$H_O$	$H_E$	$F$
my0035 (AB239390)	F: <NED> GCCCACACGACACCTACAAG R: GCCTCTCATGTACAAGTCTCAC	(CT) <sub>15</sub>	55	235–281	14	30	0.833	0.884	0.058
my0043 (AB239391)	F: <FAM> GTGTGTGACCCATTCGATTTTC R: ATTTCOCGCAACTCTCAATCT	(AG) <sub>11</sub>	55	257–275	6	32	0.844	0.771	–0.096
my0186 (AB239392)	F: <FAM> CTGCTGGTGTGTGTAGT R: GGCCTGTATTATAGAGTAGT	(AG) <sub>11</sub>	50	140–173	8	32	0.719	0.776	0.075
my0427 (AB239393)	F: <HEX> CTCGCCACACCTTCGTACTCT R: GATCGTCATGGCCGTTCTTT	(CT) <sub>15</sub>	50	225–239	8	32	0.469	0.539	0.132
my0472 (AB239394)	F: <FAM> CCTCTATATCCCTGGTACTTC R: ATTTTATCCCAATGCGTAGTCTC	(CT) <sub>10</sub>	55	101–109	4	31	0.484	0.572	0.156
my0780 (AB239395)	F: <HEX> TGGCTATCCATGACTTCTTTTTC R: GCCAAACAAGACCCAGTAAG	(CT) <sub>13</sub>	55	191–201	4	32	0.375	0.374	–0.004
my0792 (AB239396)	F: <FAM> AAAAATGTGTGCTGTGTATCT R: AATCATCATCCCATTAACCTCTC	(TG) <sub>12</sub> (CG) <sub>8</sub> (CG) <sub>2</sub> (AG) <sub>8</sub>	60	209–219	7	31	0.742	0.801	0.074
my0793 (AB239397)	F: <NED> GGGTCCTGTGTGTCTGTAAGTCTG R: TAGCGATTTAAGTTTGTCTTTGTTC	(CT) <sub>12</sub>	55	166–174	4	32	0.625	0.653	0.044
my0812 (AB239398)	F: <NED> TTGTCTTAACGCGAGGAAAGT R: CGCCAGCAATATCATTTTGT	(AG) <sub>11</sub>	50	130–138	2	32	0.281	0.365	0.231
my0841 (AB239399)	F: <FAM> GGAACATCGAACCAACAGCTAA R: CGCCGTAATCTCTCCAATAA	(AC) <sub>5</sub> (AG) <sub>10</sub>	60	113–119	4	32	0.313	0.324	0.036
my0889 (AB239400)	F: <FAM> CGCCGCAAGAAGAAGAGCACTGAT R: TCTACTGCGGTGGACCGAAGCC	(AG) <sub>10</sub>	55	181–191	6	31	0.419	0.663	0.371*
my0972 (AB239401)	F: <NED> GGAATCATCGAAGCCAGAAAA R: TAAACAAGAAATGCGAGAGGAAAG	(CT) <sub>11</sub>	55	204–224	5	31	0.645	0.723	0.109
my1001 (AB239402)	F: <FAM> TGTTCCTTTGATTCGCTCC R: TTCCCTTCTCGCTAATCGCAAGAC	(GT) <sub>17</sub>	55	134–144	12	30	0.567	0.831	0.322*

Asterisks denote significant departures from Hardy–Weinberg equilibrium. \* $P < 0.01$ .



**Figure 1.** Number and maternal origin of seeds within feces sampled in (a) late May, (b) early June, and (c) late June. The same maternal origin of seeds found in two or more feces samples are indicated by a letter (A to X) and gray shading; n indicates the number of feces samples.